(14) **Today**

Chap 3: Amino Acids, Peptides, and Proteins

Chap 4: Proteins

Next Class (15)

~~Chap 6 Enzyme Kinetics~~

Mass Spec

(16) **Second Class from Today**

Chap 6 Enzyme Kinetics

Third Class from Today (17)

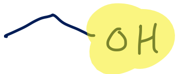
Chap 6 Enzyme Kinetics

Protein Structure: Denaturing

Section 3.2 and Chap 4

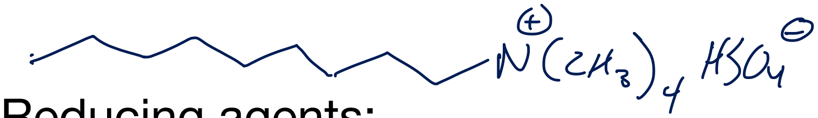
Mechanisms by which protein denature are complicated. These are some simple hypotheses about how it may occur.

1. Strong acids and bases: side chains have acid + base functional change pH changes charges, at isoelectric pt... (neutral charge)
   precipitate

2. Organic solvents: ethanol


disrupts H-bonds
   non polar solvents disrupt hydrophobic interactions

3. Detergents: interupt hydrophobic interactions



4. Reducing agents:

$$cys\}-S-S-\{cys \xrightarrow{H_2} cys\}-S-H \quad H-S-\{cys$$

5. Salt Concentration: salts compete with ionic groups for H_2O molecules
   proteins agregate + precipitate

6. Heavy Metal ions: disrupt salt bridges (+ metals take the place of the + amino acids) metals coordinate/bond to S of met cys.

7. Temperature changes ... extra energy... extra molecular motion and the non covalent interactions are not strong enough

8. Mechanical Stress

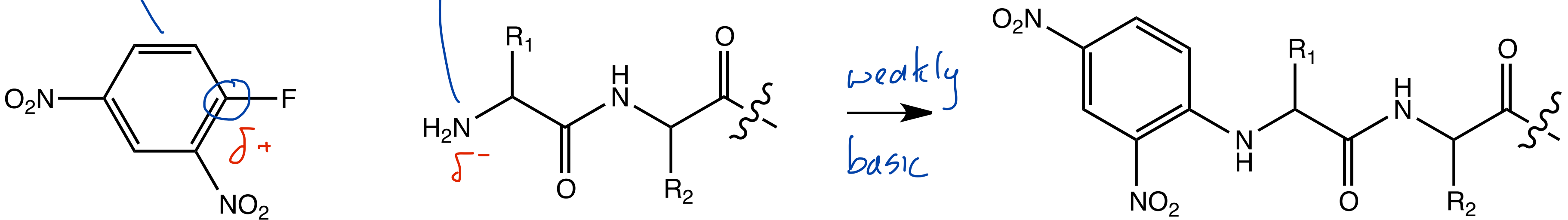
Protein Sequencing

Section 3.2 and Chap 4

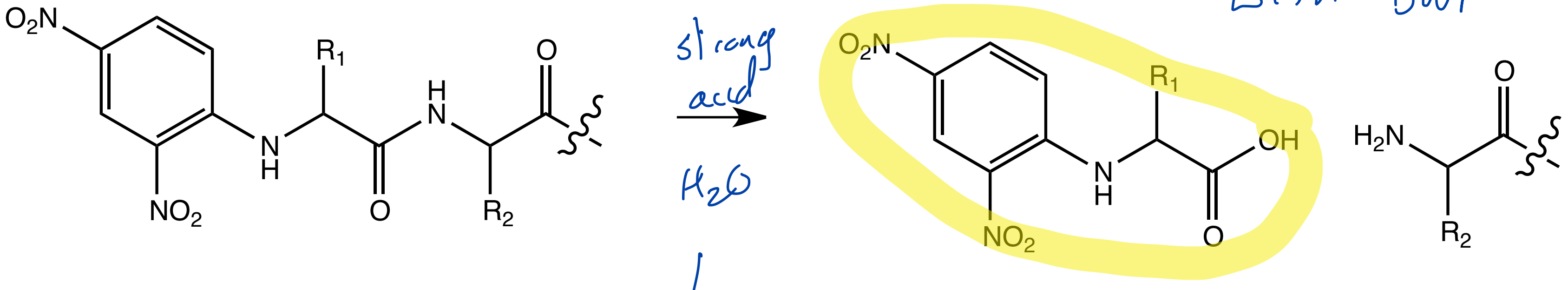
N-terminus

nucleophilic aromatic substitution

e^- rich N



end of protein tagged with DNP



hydrolyze all amide/peptide links

C-terminus

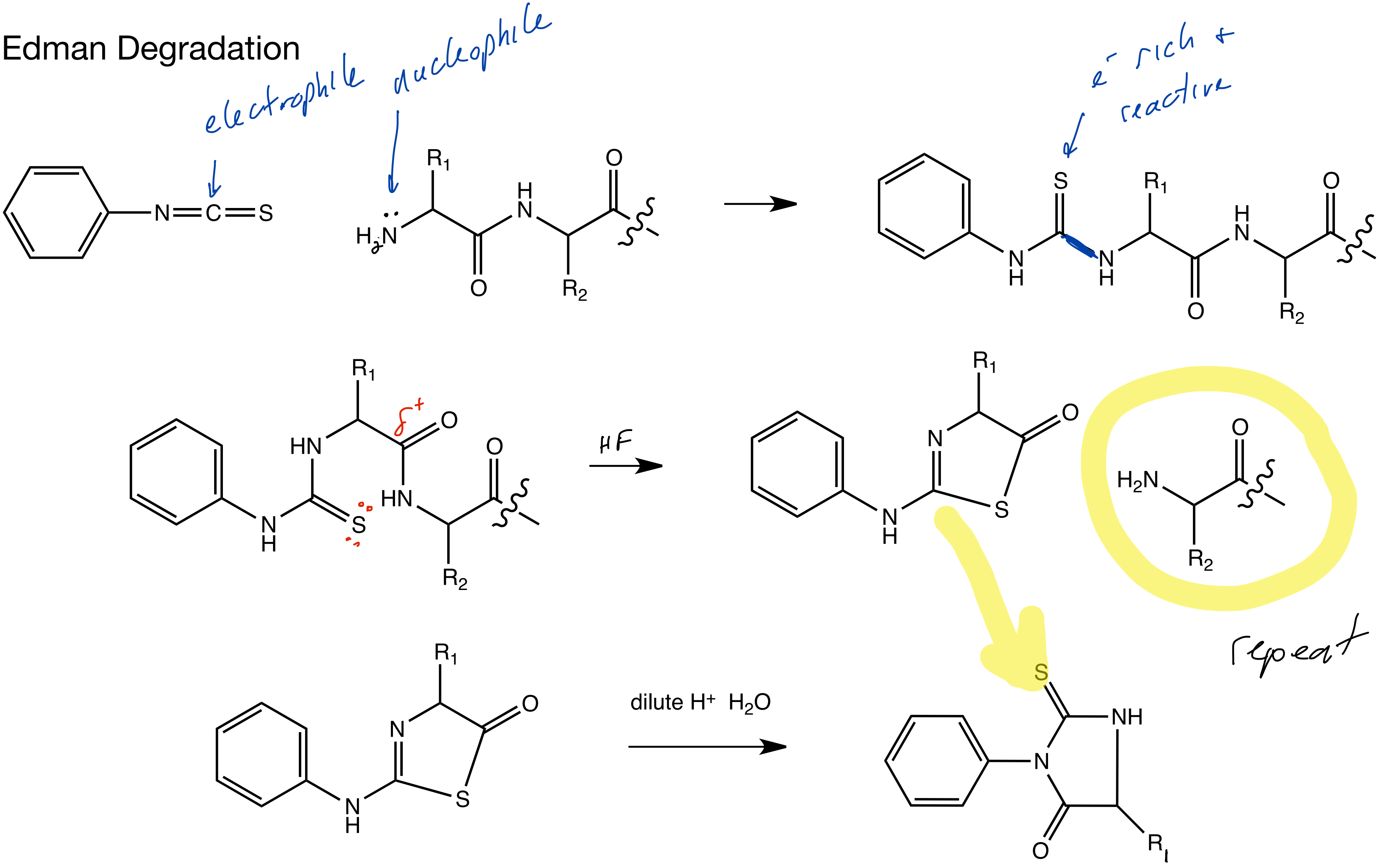
carboxy peptidase A (prefers aromatic residues) and B (prefers basic residues)

will not cleave off other amino acids

Protein Sequencing

Section 3.2 and Chap 4

Edman Degradation



good for sequences of (typically) 30 AA

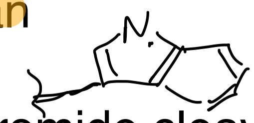
Protein Sequencing

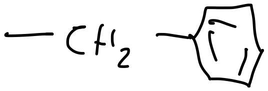
Section 3.2 and Chap 4

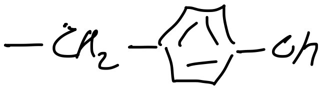
Large proteins are broken up into segments and the segments are analyzed.

trypsin cleaves residues on the carboxyl side of lysine or arginine

chymotrypsin cleaves residues on the carboxyl side of phenylalanine, tyrosine, or tryptophan







cyanogen bromide cleave peptide bonds on the carboxyl side of methionine residues

$N \equiv C-Br$

Polypeptide 1

DNP (N terminus) valine

carboxypeptidase tyrosine

treatment with trypsin gives (cleavage after lys and arginine)

ala-ala-trp-gly-lys

thr-asn-val-lys

val-leu-ser-pro-asp-lys

val-gly-ala-his-ala-gly-glu-tyr

treatment with chymotrypsin (cleavage after tryptophan, pheylalanine, tyrosine)

2

gly-lys-val-gly-ala-his-ala-gly-glu-tyr

1

val-leu-ser-pro-asp-lys-thr-asn-val-lys-ala-ala-trp

N C

V	L	S	P	A	D	K	T	N	V	K	A	A	W	G	K	V	G	A	H	A	G	E	Y

Polypeptide 2

DNP (N terminus) glu 1st AA

trypsin (cleavage after lysine and arginine)

glu-tyr-gly-ala-glu-ala-leu-glu-arg

met-phe-leu-ser-phe-pro-thr-thr-lys

thr-tyr-phe-pro-his-phe-asp-leu

chymotrypsin (cleavage after phenylalanine, tyrosine, tryptophan)

glu-tyr

pro-thr-thr-lys-thr-tyr

leu-ser-phe

pro-his-phe

phe

gly-ala-glu-ala-leu-glu-arg-met-phe

asp-leu

E	Y	G	A	E	A	L	E	R	M	F	L	S	F	P	T	T	K	T	Y	F	P	H	F	D	L

Polypeptide 3

DNP (N terminus) val

trypsin (cleavage after lysine arginine)

cys-arg

val-ala-gly-his-leu-glu-glu-tyr-gly-ala

val-lys

val-leu-ser-ser-asp-asp-lys

ala-val-trp-ser-lys

chymotrypsin (cleavage after tryptophan, tyrosine, phenylalanine)

ser-lys-val-ala-gly-his-leu-glu-glu-tyr

val-leu-ser-ser-asp-asp-lys-cys-arg-val-lys-ala-val-trp

gly-ala

V	L	S	S	D	D	K	C	N	V	K	A	V	W	S	K	V	A	G	H	L	E	E	Y	G	A